PCT/EP200 4 / 0 0 6 6 2 5

REC'D 27 JUL 2004
WIPO PCT

PA 1173184

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

BEST AVAILABLE COPY

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

May 20, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: ***60/480,245***
FILING DATE: ***June 20, 2003***

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

L. Edelen

L. EDELEN
Certifying Officer

14230 U.S. PTO
06/20/03

Docket Number	4-33201P1

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EV336719931US	June 20, 2003
Express Mail Label Number	Date of Deposit

17513 U.S. PTO
60/480245
06/20/03

Address to: Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

PATENT COVER SHEET FOR PROVISIONAL APPLICATION

Transmitted herewith for filing under 37 CFR §1.53(c) is the PROVISIONAL APPLICATION for patent of

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Klaus	Seuwen	,
Romain	Wolf	,
Marie-Gabrielle	Ludwig	,

TITLE OF THE INVENTION (280 characters max)

NOVEL G-PROTEIN COUPLED RECEPTORS AND DNA SEQUENCES THEREOF

CORRESPONDENCE ADDRESS

Direct all correspondence to the address associated with Customer No. 001095, which is currently:
Thomas Hoxie
Novartis
Corporate Intellectual Property
One Health Plaza, Building 430
East Hanover, NJ 07936-1080

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification (Including Any Claims and Abstract) - 41 pages
☐ Drawings - sheets
☒ Other (specify): Post Card

METHOD OF PAYMENT

The Commissioner is hereby authorized to charge filing fee and any additional fees required to Deposit Account Number: 19-0134 in the name of Novartis.	PROVISIONAL FILING FEE AMOUNT: $ 160

☐ U.S. Government agency and contract number: (if the invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.)

Respectfully submitted,

David E. Wildman
Attorney for Applicants
Reg. No. 40,226
Tel. No. (862) 778-7954

Date: June 20, 2003

PATENT APPLICATION SERIAL NO. ____________________

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

06/24/2003 SZEWDIE1 00000048 190134 60480245

01 FC:1005 160.00 DA

PTO-1556
(5/87)

*U.S. Government Printing Office: 2002 — 489-267/69033

Novel G-Protein Coupled Receptors and DNA Sequenc s Thereof

Field of the Invention

This invention relates to the newly identified use of proton-sensing G protein-coupled receptors (herein after referred to as "proton-sensing GPCRs") polypeptides and polynucleotides encoding such polypeptides, to their use in diagnosis and in identifying compounds that are agonists, antagonists of such proton-sensing GPCRs, and to the production of such polypeptides and polynucleotides.

Background of the Invention

In addition to its role in calcium metabolism, bone also plays a major role in regulating pH homeostasis. Bone has considerable buffering capacity that can be mobilized immediately (chemical equilibria) and slowly, by cell-mediated processes (Bushinsky DA, 2001, Eur J Nutr, 40: 238-244). In chronic a cidosis, bone resorption is increased; in contrast alkalosis tends to stimulate bone formation (Lemann J et al., 1966, J Clin Invest, 45: 1608-1614; Arnett TR et al, 1996, Bone, 18: 277-279; Bushinsky DA, 1996, Am J Physiol, 271:F216-222; Bushinsky DA, 1999, Am J Physiol, 277: F813-819). Due to suboptimal nutrition and declining renal function elderly people often present with a mild chronic acidosis, that may lead to increased bone resorption, and hence participate in the development of osteoporosis (Bushinsky DA, 2001, Eur J Nutr, 40: 238-244; Frassetto LA et al., 1996, Am J Physiol, 271: F1114-22). The pH-sensing mechanisms operating in bone cells are as yet unknown.

Summary of the Invention

The present invention is based on our surprising discovery that certain G protein-coupled receptors, in particular OGR1, act as proton-sensing receptors (proton-sensing GPCRs). Thus, the present invention relates to the novel use of certain GPRCs with proton-sensing functionality, polynucleotides encoding such polypeptides, recombinant materials and methods for their production. Such polypeptides and polynucleotides are of interest in relation to methods of treatment of certain diseases, including, but not limited to diseases and medical conditions in which proton homeostasis is altered, e.g. in diseases and medical conditions involving elevated levels of protons, i.e. hydrogen ion, including diseases of excessive bone loss, including osteoporosis, especially senile osteoporosis and osteoporosis due to renal failure. Besides bone metabolism proton-sensing GPCRs, in particular OGR1, may be involved in the regulation of respiration and cardiovascular functions and

pathological states linked to detoriation of the blood supply, like inflammation and ischemia. All of above mentioned diseases are hereinafter referred to as " diseases of the invention". In a further aspect, the invention relates to methods for identifying agonists and antagonists (e.g., inhibitors) of proton-sensing GPCRs using the materials provided by the invention, and treating conditions associated with a proton imbalance with the identified compounds. In a still further aspect, the invention relates to diagnostic assays for detecting diseases associated with inappropriate proton-sensing GPCRs activity, activities or levels.

Description of the Invention

In a first aspect, the present invention relates to a novel use of certain GPRC polypeptides in pH homeostasis.

Such polypeptides are selected from one of the groups consisting of: :

(a) an isolated polypeptide encoded by a polynucleotide comprising the polynucleotide sequence of human OGR1 (accession: NM_003485.1), rat OGR1 (accession: XM_234483), mouse OGR1 (accession: NM_175493), bovine OGR1 (accession: NM_174329), preferably human OGR1 (accession: NM_003485.1);

(b) an isolated polypeptide comprising a polypeptide sequence having at least 80%, preferably 85%, more preferably 90%, more preferably 95%, more preferably 96%, more preferably 97%, more preferably 98%, or more preferably 99% identity to the polypeptide sequence of SEQ ID NO: 1;

(c) an isolated polypeptide comprising the polypeptide sequence of SEQ ID NO: 1;

(d) an isolated polypeptide having at least 80%, preferably 85%, more preferably 90%, more preferably 95%, more preferably 96%, more preferably 97%, more preferably 98%, or more preferably 99% identity to the polypeptide sequence of SEQ ID NO: 1;

(e) the polypeptide sequence of SEQ ID NO: 1;

(f) an isolated polypeptide having or comprising a polypeptide sequence that has an Identity Index of 0.8, preferably 0.85, more preferably 0.95, more preferably 0.96, more preferably 0.97, more preferably 0.98, or more preferably 0.99 compared to the polypeptide sequence of SEQ ID NO: 1;

(g) fragments and variants of such polypeptides in (a) to (f); and

(h) polypeptides in (a) to (f) which show a pH dependent Inositol phosphate formation in CCL39 hamster fibroblast cells.

Polypeptides of the present invention are members of the G protein-coupled receptors family of polypeptides. The biological properties of the proton-sensing GPCRs polypeptides as defined above and in the Experimental part are hereinafter referred to as "biological activity or activities of the proton-sensing GPCRs" or "proton-sensing activity". Preferably, a polypeptide of the present invention exhibits at least one biological activity of the proton-sensing GPCRs as defined above. More preferably, a polypeptide of the present invention exhibits at least one biological activity of OGR1.

Polypeptides of the present invention also includes variants of the aforementioned polypeptides, including all allelic forms and splice variants. Such polypeptides vary from the reference polypeptide by insertions, deletions, and substitutions that may be conservative or non-conservative, or any combination thereof. Particularly preferred variants are those in which several, for instance from 50 to 30, from 30 to 20, from 20 to 10, from to 5, from 5 to 3, from 3 to 2, from 2 to 1 or 1 amino acids are inserted, substituted, or deleted, in any combination.

Preferred fragments of polypeptides of the present invention include an isolated polypeptide comprising an amino acid sequence having at least 30, 50 or 100 contiguous amino acids from the amino acid sequence of SEQ ID NO: 1, or an isolated polypeptide comprising an amino acid sequence having at least 30, 50 or 100 contiguous amino acids truncated or deleted from the amino acid sequence of SEQ ID NO: 1. Preferred fragments are biologically active fragments that block or enhance the biological activity of GPCRs of the invention, in particular OGR1, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also preferred are those fragments that are antigenic or immunogenic in an animal, especially in a human.

Fragments of the polypeptides of the invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, these variants may be employed as intermediates for producing the full-length polypeptides of the invention. The polypeptides of the present invention may be in the form of the "mature" protein or maybe a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence that contains secretory or leader sequences, pro-sequences, sequences that aid in purification, for instance multiple histidine residues, or an additional sequence for stability during recombinant production.

Polypeptides of the present invention can be prepared in any suitable manner, for instance by isolation form naturally occurring sources, from genetically engineered host cells comprising expression systems (vide infra) or by chemical synthesis, using for instance automated peptide synthesizers, or a combination of such methods. The means for preparing such polypeptides are well understood in the art.

In a further aspect, the present invention relates to a novel use of certain GPCR polynucleotides in pH homeostasis. Such polynucleotides are selected from one of the groups consisting of:
(a) an isolated polynucleotide comprising a polynucleotide sequence having at least 80%, preferably 85%, more preferably 90%, more preferably 95%, more preferably 96%, more preferably 97%, more preferably 98%, or more preferably 99% identity to the polynucleotide sequence of human OGR1 (accession: NM_003485.1);
(b) an isolated polynucleotide comprising the polynucleotide of human OGR1 (accession: NM_003485.1);
(c) an isolated polynucleotide having at least 80%, preferably 85%, more preferably 95%, more preferably 96%, more preferably 97%, more preferably 98%, or more preferably 99% identity to the polynucleotide of human OGR1 (accession: NM_003485.1);
(d) the isolated polynucleotide of human OGR1 (accession: NM_003485.1);
(e) an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 80%, preferably 85%, more preferably 90%, more preferably 95%, more preferably 96%, more preferably 97%, more preferably 98%, or more preferably 99% identity to the polypeptide sequence of SEQ ID NO: 1;
(f) an isolated polynucleotide comprising a polynucleotide sequence encoding the polypeptide of SEQ ID NO:1;
(g) an isolated polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 80%, preferably 85%, more preferably 90%, more preferably 95%, more preferably 96%, more preferably 97%, more preferably 98%, or more preferably 99% identity to the polypeptide sequence of SEQ ID NO:1;
(h) an isolated polynucleotide encoding the polypeptide of SEQ ID NO:1;
(i) an isolated polynucleotide having or comprising a polynucleotide sequence that has an Identity Index of 0.8, preferably 0.85, more preferably 0.9, more preferably 0.95, more

preferably 0.96, more preferably 0.97, more preferably 0.98, or more preferably 0.99 compared to the polynucleotide sequence of human OGR1 (accession: NM_003485.1);
(k) an isolated polynucleotide having or comprising a polynucleotide sequence encoding a polypeptide sequence that has an Identity Index of 0.8, preferably 0.85, more preferably 0.9, more preferably 0.95, more preferably 0.96, more preferably 0.97, more preferably 0.98, or more preferably 0.99 compared to the polypeptide sequence of SEQ ID NO:1; and polynucleotides that are fragments and variants of the above mentioned polynucleotides or that are complementary to above mentioned polynucleotides, over the entire length thereof; and
(i) polynucleotides in (a) to (k) that encode for polypeptides that show a pH dependent Inositol phosphate formation in CCL39 hamster fibroblast cells.

Preferred fragments of polynucleotides for use in modulating pH homeostasis include an isolated polynucleotide comprising an nucleotide sequence having at least 15, 30, 50 or 100 contiguous nucleotides from the sequence of human OGR1 (accession: NM_003485.1) or an isolated polynucleotide comprising an sequence having at least 30, 50 or 100 contiguous nucleotides truncated or deleted from the sequence of human OGR1 (accession: NM_003485.1).

Preferred variants of polynucleotides for use in modulating pH homeostasis include splice variants, allelic variants, and polymorphisms, including polynucleotides having one or more single nucleotide polymorphisms (SNPs).

Polynucleotides for use in modulating pH homeostasis also include polynucleotides encoding polypeptide variants that comprise the amino acid sequence of SEQ ID NO: 1 and in which several, for instance from 50 to 30, from 30 to 20, from 20 to 10, from 10 to 5, from 5 to 3, from 3 to 2, from 2 to 1 or 1 amino acid residues are substituted, deleted or added, in any combination.

In a further aspect, the present invention provides polynucleotides for use in modulating pH homeostasis that are RNA transcripts of the DNA sequences of the present invention. Accordingly, there is provided an RNA polynucleotide for use in modulating pH homeostasis that:

(a) comprises an RNA transcript of the DNA sequence encoding the polypeptide of SEQ ID NO: 1;
(b) is the RNA transcript of the DNA sequence encoding the polypeptide of SEQ ID NO: 1;
(c) comprises an RNA transcript of the DNA sequence of human OGR1 (accession: NM_003485.1); or
(d) is the RNA transcript of the DNA sequence of human OGR1 (accession: NM_003485.1); and RNA polynucleotides that are complementary thereto.

The polynucleotide sequence of human OGR1 (accession: NM_003485.1) is a cDNA sequence that encodes the polypeptide of SEQ ID NO: 1. The polynucleotide sequence encoding the polypeptide of SEQ ID NO: 1 may be identical to the polypeptide encoding sequence of human OGR1 (accession: NM_003485.1) or it may- be a sequence other than human OGR1 (accession: NM_003485.1) which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO: 1.

Polynucleotides for use in modulating pH homeostasis may be obtained using standard cloning and screening techniques from a cDNA library derived from mRNA in e.g. brain, kidney, lung and cells of the immune system (for expression of OGR1 see e.g. Xu et al., 2000, Nat. Cell Biol. 2, 261-267; Zhu et al., 2001, J. Biol. Chem. 276, 41325-41335; Xu et al., 1996, Genomics 35, 397-402) (for standard cloning technique see for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

When polynucleotides of the present invention are used for the recombinant production of polypeptides for use in modulating pH homeostasis, the polynucleotide may include the coding sequence for the mature polypeptide, by itself, or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence that facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et aL, Proc Natl Acad Sci USA (1989) 86:821-824, or

is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

A polynucleotide encoding a polypeptide of the present invention, including homologs from species not yet known, may be obtained by a process comprising the steps of screening a library under stringent hybridization conditions with a labelled probe having the sequence of SEQ ID NO: 1 or a fragment thereof, preferably of at least 15 nucleotides; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan. Preferred stringent hybridization conditions include overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA; followed by washing the filters in O.1x SSC at about 65°C. Thus the present invention also includes isolated polynucleotides for use in modulating pH homeostasis, preferably with a nucleotide sequence of at least 100, obtained by screening a library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO: 1 or a fragment thereof, preferably of at least 15 nucleotides.

The person skilled in the art will appreciate that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide does not extend all the way through to the 5' terminus. This is a consequence of reverse transcriptase, an enzyme with inherently low "processivity" (a measure of the ability of the enzyme to remain attached to the template during the polymerisation reaction), failing to complete a DNA copy of the mRNA template during first strand cDNA synthesis.

There are several methods available and well known to those skilled in the art to obtain full-length cDNAs, or extend short cDNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman et al., Proc Nat Acad Sci USA 85, 8998- 9002, 1988). Recent modifications of the technique, exemplified by the Marathon (trade mark) technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon (trade mark) technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried

out to amplify the "missing" 5' end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using 'nested' primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence). The products of this reaction can then be analysed by DNA sequencing and a full-length cDNA constructed either by joining the product directly to the existing cDNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

Recombinant polypeptides of the present invention may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, in a further aspect, the present invention relates to expression systems comprising a polynucleotide or polynucleotides of the present invention, to host cells which are genetically engineered with such expression systems and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Polynucleotides may be introduced into host cells by methods described in many standard laboratory manuals, such as Davis et al., Basic Methods in Molecular Biology (1986) and Sambrook et al.(ibid).

Preferred methods of introducing polynucleotides into host cells include, for instance, calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as Streptococci, Staphylococci, E coli, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and Aspergillus cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C1 27, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used, for instance, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector that is able to maintain, propagate or express a polynucleotide to produce a polypeptide in a host may be used. The appropriate polynucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al (see above). Appropriate secretion signals may be incorporated into the desired polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and/or purification.

Polynucleotides of the present invention may be used as diagnostic reagents, through detecting mutations in the associated gene. Detection of a mutated form of the gene characterised by the polynucleotide of human OGR1 (accession: NM_003485.1) in the cDNA or genomic sequence and which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered spatial or temporal expression of

the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques well known in the art.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or it may be amplified enzymatically by using PCR, preferably RT-PCR, or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled OGR1 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures.

DNA sequence difference may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (see, for instance, Myers et aL, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S 1 protection or the chemical cleavage method (see Cotton et aL, Proc Natl Acad Sci USA (1985) 85: 4397-4401).

An array of oligonucleotides probes comprising OGR1 polynucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Such arrays are preferably high density arrays or grids. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability, see, for example, M.Chee et al., Science, 274, 610- 613 (1996) and other references cited therein.

Detection of abnormally decreased or increased levels of polypeptide or mRNA expression may also be used for diagnosing or determining susceptibility of a subject to a disease of the invention. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT- PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as a polypeptide of the present invention, in a sample derived from

a host are well-known to those skilled in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit comprising:
(a) a polynucleotide of the present invention, preferably the nucleotide sequence of human OGR1 (accession: NM_003485.1), or a fragment or an RNA transcript thereof;
(b) a nucleotide sequence complementary to that of (a);
(c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO: 1 or a fragment thereof; or
(d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO: 1.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a disease, particularly diseases of the invention, amongst others.

The polypeptides of the present invention are expressed in e.g. brain, kidney, lung and cells of the immune system (Xu et al., 2000, Nat. Cell Biol. 2, 261-267; Zhu et al., 2001, J. Biol. Chem. 276, 41325-41335; Xu et al., 1996, Genomics 35, 397-402).

A further aspect of the present invention relates to antibodies. The polypeptides of the invention or their fragments, or cells expressing them, can be used as immunogens to produce antibodies that are immunospecific for polypeptides of the present invention. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art. Antibodies generated against polypeptides of the present invention may be obtained by administering the polypeptides or epitope-bearing fragments, or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et aL, Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole et aL, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in U.S. Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography. Antibodies against polypeptides of the present invention may also be employed to treat diseases of the invention, amongst others.

Polypeptides and polynucleotides of the present invention may also be used as vaccines. Accordingly, in a further aspect, the present invention relates to a method for inducing an immunological response in a mammal that comprises inoculating the mammal with a polypeptide of the present invention, adequate to produce antibody and/or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said animal from disease, whether that disease is already established within the individual or not. An immunological response in a mammal may also be induced by a method comprises delivering a polypeptide of the present invention via a vector directing expression of the polynucleotide and coding for the polypeptide in vivo in order to induce such an immunological response to produce antibody to protect said animal from diseases of the invention. One way of administering the vector is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a modified nucleic acid, or a DNA/RNA hybrid. For use a vaccine, a polypeptide or a nucleic acid vector will be normally provided as a vaccine formulation (composition). The formulation may further comprise a suitable carrier. Since a polypeptide may be broken down -in the stomach, it is preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions that may contain anti-oxidants, buffers, bacteriostats and solutes that render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions that may include suspending agents or thickening agents.

The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze- dried condition requiring only the

addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Polypeptides of the present invention have one or more biological functions that are of relevance in one or more disease states, in particular the diseases of the invention hereinbefore mentioned. It is therefore useful to identify compounds that stimulate or inhibit the function or level of the polypeptide. Accordingly, in a further aspect, the present invention provides for a method of screening compounds to identify those that stimulate or inhibit the function or level of the polypeptide. Such methods identify agonists or antagonists that may be employed for therapeutic and prophylactic purposes for such diseases of the invention as hereinbefore mentioned. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, collections of chemical compounds, and natural product mixtures. Such agonists or antagonists so-identified may be natural or modified substrates, ligands, receptors, enzymes, etc., as the case may be, of the polypeptide; a structural or functional mimetic thereof (see Coligan et aL, Current Protocols in Immunology 1(2):Chapter 5 (1991)) or a small molecule.

The screening method may simply measure the binding of a candidate compound to the polypeptide, or to cells or membranes bearing the polypeptide, or a fusion protein thereof, by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve measuring or detecting (qualitatively or quantitatively) the competitive binding of a candidate compound to the polypeptide against a labeled competitor (e.g. agonist or antagonist). Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells bearing the polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide of the present invention, to form a mixture, measuring a HGRL101 activity in the mixture, and comparing the HGRL101 activity of the mixture to a control mixture which contains no candidate compound.

Polypeptides of the present invention may be employed in conventional low capacity screening methods and also in high-throughput screening (HTS) formats. Such HTS formats include not only the well-established use of 96- and, more recently, 384-well microtiter plates but also emerging methods such as the nanowell method described by Schullek et al, Anal Biochem., 246, 20-29, (1997).

Fusion proteins, such as those made from Fc portion and OGR1 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists for the polypeptide of the present invention (see D. Bennett et aL, J Mol Recognition, 8:52-58 (1995); and K. Johanson et al., J Biol Chem, 270(16):9459-9471 (1995)).

Screening techniques

The polynucleotides, polypeptides and antibodies to the polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents that may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

A polypeptide of the present invention may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the polypeptide is labeled with a radioactive isotope (for instance, ^{125}I), chemically modified (for instance, biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. These screening methods may also be used to identify agonists and antagonists of the polypeptide that compete with the binding of the polypeptide to its receptors, if any. Standard methods for conducting such assays are well understood in the art.

Examples of antagonists of polypeptides of the present invention include antibodies or, in some cases, oligonucleotides or proteins that are closely related to the ligands, substrates, receptors, enzymes, etc., as the case may be, of the polypeptide, e.g., a fragment of the ligands, substrates, receptors, enzymes, etc.; or a small molecule that bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Screening methods may also involve the use of transgenic technology. The art of constructing transgenic animals is well established. For example, the OGR1 gene may be introduced through microinjection into the male pronucleus of fertilized oocytes, retroviral transfer into pre- or post-implantation embryos, or injection of genetically modified, such as by electroporation, embryonic stem cells into host blastocysts. Particularly useful transgenic animals are so- called "knock-in" animals in which an animal gene is replaced by the human equivalent within the genome of that animal. Knock-in transgenic animals are useful in the drug discovery process, for target validation, where the compound is specific for the human target. Other useful transgenic animals are so-called "knock-out" animals in which the expression of the animal ortholog of a polypeptide of the present invention and encoded by an endogenous DNA sequence in a cell is partially or completely annulled. The gene knock-out may be targeted to specific cells or tissues, may occur only in certain cells or tissues as a consequence of the limitations of the technology, or may occur in all, or substantially all, cells in the animal. Transgenic animal technology also offers a whole animal expression-cloning system in which introduced genes are expressed to give large amounts of polypeptides of the present invention.

Screening kits for use in the above described methods form a further aspect of the present invention. Such screening kits comprise:

(a) a polypeptide of the present invention;

(b) a recombinant cell expressing a polypeptide of the present invention,

(c) a cell membrane expressing a polypeptide of the present invention; or

(d) an antibody to a polypeptide of the present invention; which polypeptide is preferably that of SEQ ID NO: 1.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Glossary

The following definitions are provided to facilitate understanding of certain terms used frequently hereinbefore.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered by the human hands from its natural state, ie. if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide" generally refers to any polyribonucleotide (RNA) or polydeoxribonucleotide (DNA), which may be unmodified or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double- stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

"Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any polypeptide comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, - i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini.
It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutarnate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, Proteins - Structure and Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, 1-12, in Post-translational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et aL, "Analysis for protein modifications and nonprotein cofactors", Meth Enzymol, 182, 626-646, 1990, and Rattan et al., "Protein Synthesis: Post-translational Modifications and Aging", Ann NY Acad Sci, 663, 48-62, 1992).

"Fragment" of a polypeptide sequence refers to a polypeptide sequence that is shorter than the reference sequence but that retains essentially the same biological function or activity as the reference polypeptide of SEQ ID NO: 2. "Fragment" of a polynucleotide sequence refers to a polynucloetide sequence that is shorter than the reference sequence of SEQ ID NO: 1.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains the essential properties thereof. A typical variant of a polynucleotide differs in nucleotide sequence from the reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from the reference polypeptide. Generally, alterations are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, insertions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. Typical conservative substitutions include Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln-l Ser, Thr; Lys, Arg; and Phe and Tyr. A variant of a polynucleotide or polypeptide may be naturally occurring such as an allele, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Also included as variants are polypeptides having one or more post-translational modifications, for instance glycosylation, phosphorylation, methylation, ADP ribosylation and the like. Embodiments include methylation of the N-terminal amino acid, phosphorylations of serines and threonines and modification of C- terminal glycines.

"Allele" refers to one of two or more alternative forms of a gene occuring at a given locus in the genome.

"Polymorphism" refers to a variation in nucleotide sequence (and encoded polypeptide sequence, if relevant) at a given position in the genome within a population.

"Singl Nucleotid Polymorphism" (SNP) refers to the occurence of nucleotide variability at a single nucleotide position in the genome, within a population. An SNP may occur within a gene or within intergenic regions of the genome. SNPs can be assayed using Allele Specific Amplification (ASA). For the process at least 3 primers are required. A common primer is used in reverse complement to the polymorphism being assayed. This common primer can be between 50 and 1500 bps from the polymorphic base. The other two (or more) primers are identical to each other except that the final 3' base wobbles to match one of the two (or more) alleles that make up the polymorphism. Two (or more) PCR reactions are then conducted on sample DNA, each using the common primer and one of the Allele Specific Primers.

"Splice Variant" as used herein refers to cDNA molecules produced from RNA molecules initially transcribed from the same genomic DNA sequence but which have undergone alternative RNA splicing. Alternative RNA splicing occurs when a primary RNA transcript undergoes splicing, generally for the removal of introns, which results in the production of more than one mRNA molecule each of that may encode different amino acid sequences. The term splice variant also refers to the proteins encoded by the above cDNA molecules.

"Identity" reflects a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, determined by comparing the sequences. In general, identity refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of the two polynucleotide or two polypeptide sequences, respectively, over the length of the sequences being compared.

"% Identity" - For sequences where there is not an exact correspondence, a "% identity" may be determined. In general, the two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may be determined over the whole length of each of the sequences being - compared (so-called global alignment), that is particularly suitable for sequences of the same or very similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length.

"Similarity" is a further, more sophisticated measure of the relationship between two polypeptide sequences. In general, "similarity" means a comparison between the amino acids of two polypeptide chains, on a residue by residue basis, taking into account not only exact correspondences between pairs of residues, one from each of the sequences being compared (as for identity) but also, where there is not an exact correspondence, whether, on an evolutionary basis, one residue is a likely substitute for the other. This likelihood has an associated "score" from which the "% similarity" of the two sequences can then be determined.

Methods for comparing the identity and similarity of two or more sequences are well known in the art. Thus for instance, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux J et al, Nucleic Acids Res, 12, 387-395, 1984, available from Genetics Computer Group, Madison, Wisconsin, USA), for example the programs BESTFIT and GAP, may be used to determine the % identity between two polynucleotides and the % identity and the % similarity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of Smith and Waterman (J Mol Biol, 147,195-197, 1981, Advances in Applied Mathematics, 2, 482-489, 1981) and finds the best single region of similarity between two sequences. BESTFIT is more suited to comparing two polynucleotide or two polypeptide sequences that are dissimilar in length, the program assuming that the shorter sequence represents a portion of the longer. In comparison, GAP aligns two sequences, finding a "maximum similarity", according to the algorithm of Neddleman and Wunsch (J Mol Biol, 48, 443-453, 1970). GAP is more suited to comparing sequences that are approximately the same length and an alignment is expected over the entire length. Preferably, the parameters "Gap Weight" and "Length Weight" used in each program are 50 and 3, for polynucleotide sequences and 12 and 4 for polypeptide sequences, respectively. Preferably, % identities and similarities are determined when the two sequences being compared are optimally aligned.

Other programs for determining identity and/or similarity between sequences are also known in the art, for instance the BLAST family of programs (Altschul S F et al, J Mol Biol, 215, 403-410, 1990, Altschul S F et al, Nucleic Acids Res., 25:389-3402, 1997, available from the National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA and accessible through the home page of the NCBI at www.ncbi.nlm.nih.gov) and FASTA (Pearson W R, Methods in Enzymology, 183, 63-99, 1990; Pearson W R and Lipman D J,

Proc Nat Acad Sci USA, 85, 2444-2448,1988, available as part of the Wisconsin Sequence Analysis Package).

Preferably, the BLOSUM62 amino acid substitution matrix (Henikoff S and Henikoff J G, Proc. Nat. Acad Sci. USA, 89, 10915-10919, 1992) is used in polypeptide sequence comparisons including where nucleotide sequences are first translated into amino acid sequences before comparison.

Preferably, the program BESTFIT is used to determine the % identity of a query polynucleotide or a polypeptide sequence with respect to a reference polynucleotide or a polypeptide sequence, the query and the reference sequence being optimally aligned and the parameters of the program set at the default value, as hereinbefore described.

"Identity Index" is a measure of sequence relatedness which may be used to compare a candidate sequence (polynucleotide or polypeptide) and a reference sequence. Thus, for instance, a candidate polynucleotide sequence having, for example, an Identity Index of 0.95 compared to a reference polynucleotide sequence is identical to the reference sequence except that the candidate polynucleotide sequence may include on average up to five differences per each 100 nucleotides of the reference sequence. Such differences are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion. These differences may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between these terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polynucleotide sequence having an Identity Index of 0.95 compared to a reference polynucleotide sequence, an average of up to 5 - 25 in every 100 of the nucleotides of the in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies mutatis mutandis for other values of the Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.

Similarly, for a polypeptide, a candidate polypeptide sequence having, for example, an Identity Index of 0.95 compared to a reference polypeptide sequence is identical to the reference sequence except that the polypeptide sequence may include an average of up to five differences per each 100 amino acids of the reference sequence. Such differences are

selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion. These differences may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between these terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. In other words, to obtain a polypeptide sequence having an Identity Index of 0.95 compared to a reference polypeptide sequence, an average of up to 5 in every 100 of the amino acids in the reference sequence may be deleted, substituted or inserted, or any combination thereof, as hereinbefore described. The same applies mutatis mutandis for other values of the Identity Index, for instance 0.96, 0.97, 0.98 and 0.99.
The relationship between the number of nucleotide or amino acid differences and the

Identity Index may be expressed in the following equation:

$$n_a \leq x_a - (x_a \bullet I)$$

in which:

n_a is the number of nucleotide or amino acid differences,
x_a is the total number of nucleotides or amino acids in SEQ ID NO: 1 or SEQ ID NO: 2, respectively,
I is the Identity Index,
$\bullet$ is the symbol for the multiplication operator, and in which any non-integer product Of x_a and I is rounded down to the nearest integer prior to subtracting it from x_a.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a reference sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the two sequences as hereinbefore defined. Falling within this generic term are the terms "ortholog", and "paralog". "Ortholog" refers to a polynucleotide or polypeptide that is the functional equivalent of the polynucleotide or polypeptide in another species. "Paralog" refers to a polynucleotideor polypeptide that within the same species which is functionally similar.

"Fusion protein" refers to a protein encoded by two, unrelated, fused genes or fragments thereof. Examples have been disclosed in US 5541087, 5726044.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

Examples

Example 1: Cell culture and transfections:

CCL39 hamster fibroblasts, HEK 293 human embryonic kidney cells, and MG63 human osteosarcoma cells (all cell lines from ATCC = American type culture collection , Manassas, USA) are grown in a 1:1 mixture of bicarbonate-buffered DMEM and Ham's F12 medium supplemented with 10% fetal calf serum and antibiotics, in CO_2 atmosphere at pH 7.4. Primary cultures of human trabecular bone – derived preosteoblasts are established and cultured as described in detail before (Sottile et al., 2002, *Bone* **30**, 699-704) Expression vectors for human OGR1 are prepared by cloning the cDNA of this receptor from human genomic DNA (U48405, NM_003485) into pcDNA3.1(+)/myc-His (Invitrogen, Basel, Switzerland). Site-directed mutagenesis is carried out using the Quick Change kit from Stratagene (Basel, Switzerland). For stable transfection, vectors are linearised with PvuI. Stable and transient transfections are carried out using the Effectene reagent (Qiagen, Basel, Switzerland). Stable cell populations expressing receptors are isolated following selection with antibiotic G418 (400 µg/ml). Expression of transgenes and membrane localisation is verified by performing immunocytochemistry using a FITC-labelled anti-myc antibody (Zymed/Stehelin & Cie, Basel).

Example 2: OGR1 is a proton-sensing G protein coupled receptor activating IP formation

Inositol phosphate (IP) formation assay: Buffers and pH: Salt solutions for IP formation experiments are buffered either with HEPES alone (20 mM) or HEPES/EPPS/MES (8 mM each), to cover a wider pH range. HEPES is 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, EPPS is N'-(2-hydroxyethyl)piperazine-N'-3-propanesulfonic acid, MES is 2-(N-morpholino)ethanesulfonic acid. The pH of all solutions is adjusted to the indicated value at room temperature using a carefully calibrated pH meter (Metrohm, Herisau, Switzerland). All data in this report are referenced to pH at room temperature. To obtain pH at 37°C, 0.15 pH units should be subtracted for HEPES buffers in the range of pH 6.8 – 7.8 according to our calibration experiments. IP formation assay. Confluent cell cultures grown in 24 well plates are labelled with myo[^{3}H]inositol (100 MBq/ml; ART/Anawa Trading, Wangen-Duebendorf, Switzerland) for 24h in serum-free DMEM medium. Cells are then incubated at 37°C in a buffered salt solution containing 130 mM NaCl, 0.9 mM NaH_2PO_4, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 25 mM glucose. Lithium (20 mM) is added to block inositol monophosphatase activity, leading to accumulation of IP_1 (Berridge et al., 1982, Biochem j. 206, 587-595; Berridge et Irvine, 1989, Nature 341, 197-205). Where indicated, bovine

thrombin or SPC (both from Sigma, Buchs, Switzerland) is added 1 min prior to lithium addition. Unless otherwise stated, incubation is continued for 20 minutes. Cells are then extracted with ice-cold formic acid and total IPs separated from free inositol using batch column chromatography exactly as described before (Seuwen et al, 1988, EMBO J 7, 161-168).

Results: Following transient or stable expression of OGR1 a robust and apparently ligand-independent activation of phosphoinositide turnover in transfected cells is noticed. This effect persists in assay buffer devoid of either calcium, magnesium, phosphate, or sulfate, excluding that OGR1 is activated by any of these constituents. However, variations of buffer pH strongly affects inositol phosphate (IP) formation:

CCL39 hamster fibroblasts stably expressing OGR1 are incubated in Hepes-buffered salt solution at different pH, and accumulation of IPs measured at 37°C in the absence or presence of the inositol monophosphatase inhibitor lithium (Berridge et al., 1982, Biochem j. 206, 587-595; Berridge et Irvine, 1989, Nature 341, 197-205). At pH 7.6, IP formation in the presence of lithium is close to background, however, lower pH resulted in a significant accumulation of IPs, which is linear over 30 minutes and maximal at pH 6.8. The pH of our standard assay buffer is 7.4, explaining why a significant basal activity has been observed in previous experiments. Activation of OGR1 by protons can be plotted as a standard concentration-response curve, covering a wider range of extracellular pH. Half-maximal activation of the receptor expressed in CCL39 hamster fibroblasts occurs at pH 7.48 +/- 0.04 (mean +/- sem; N=12). Activation appears highly co-operative, with a Hill coefficient of >2. Under acidic conditions (pH <6.5), IP formation declines again, reflecting the general pH-dependence of the phosphoinositide signalling system in CCL39 cells. SPC, the bioactive lipid reported to activate OGR1, does not stimulate the receptor in our hands, and does not affect pH-dependent stimulation of IP formation. The molecule is active, however, inhibiting forskolin-stimulated adenylyl cyclase in other cell systems, suggesting it acts on an as yet undefined receptor distinct from OGR1.

Importantly, untransfected cells or cells expressing other receptors do not show pH-dependent stimulation of phosphoinositide turnover. Similarly, the response to other receptor agonists is not positively modulated in the range of pH 7-8, as demonstrated here for thrombin, which activates endogenous receptors in CCL39 cells (Paris et Pouyssegur, 1086, EMBO J. 5, 55-60). Pertussis toxin (PTX) does not inhibit IP formation measured in the presence of lithium at pH 7, strongly suggesting that OGR1 activates phosphoinositide turnover through Gq. The response to thrombin, which is additive to the signal elicited by

OGR1 in these cells, is partially inhibited by PTX both at pH 7 and at pH 7.6, as expected from earlier work (Paris et Pouyssegur, 1086, EMBO J. 5, 55-60).

Activation of OGR1 at neutral or slightly acidic pH is very strong, comparable to activation of other GPCRs by their cognate ligands. Strong pH-dependent activation of IP formation is also observed in transiently transfected HEK cells (half-maximal activation at pH 7.38 +/- 0.04 (mean +/- sem; N=4)) and in stably transfected HEK cells. Some pH-activated channels are strongly sensitive to temperature (Smith et al., 2002, Nature 418, 186-190). However, IP formation rate for OGR1 measured at pH 7.6 and pH 7.0 is only minimally affected by temperature in the range of 35 – 42°C.

<u>Example 2: Receptor model of OGR1</u>

Receptor model: Given the high similarity between OGR1 and other rhodopsin-like GPCRs the published rhodopsin structure (Palczewski et al., 2000, Science 289, 739-745) as a template to build a homology model. The OGR1 primary sequence is aligned to bovine rhodopsin (bRHO), and for the indicated amino acid positions the side chains present in bRHO are substituted by the corresponding side chains of OGR1, using the SCWRL program (Bower et al., 1997, J. Mol. Biol. 267, 1268-1282). The resulting structure is refined applying molecular mechanics and dynamics, using the parm96.dat parameters of the AMBER force field (Cornell et al., 1995, J. Am. Chem. Soc. 117, 5179-5197) with the distance-dependent dielectric function of epsilon = 1*r, the minimax module of our own Wit!P software for energy refinements, and the sander_classic module of the AMBER6 package. During all computations, the Calpha atoms of the template are constrained in motion by fixing them with a harmonic potential or by constraining their position within 0.5 A (potential energy minimizations). The extracellular loops and all amino acid side chains are left free to move within the potential of the force field. A disulphide bond is introduced between residues CYS94 and CYS172. Intracellular loops are not included.

Results: Histidines should play an important role in the pH-dependent activation of OGR1. Indeed, inspection of a 3D model of the receptor indicates that several histidines might cluster at the extracellular surface, placed on top of helices I, IV and VII, and in extracellular loops 1 and 2. All are conserved in the mouse, rat, and bovine sequence (see accession nos. XM_138218, XM_234483 and U88367, respectively). Specifically, the model predicts a direct hydrogen bond interaction between H20 and H269 in the unprotonated state, thus linking helix I and helix VII. A second interaction is possible between H17 and H84, linking the receptor N terminus to extracellular loop 1. However, given the flexibility of the

extracellular loops, this interaction appears less likely to form than the interaction between H20 and H269, and initial constraints are required in our model to make it fall in place. Additional histidines are found at other locations in the receptor, but their mutual interaction or strong electrostatic interactions with other residues seemed less obvious. We set out to mutate all potentially critical histidines individually to phenylalanine, and to measure pH-dependent receptor activation in transient transfection experiments. All receptor constructs are screened for expression of the recombinant protein by immunocytochemistry, using the C-terminal myc tag, and found to be expressed on the cell membrane at similar levels. Mutation of histidines 89,159,175 remains without major effect on receptor function. However, in agreement with our expectations, histidines 17,20,84,269 are each required for normal sensitivity to pH change. In addition H169 turned out to be important. Mutation of these amino acids results in receptors that failed to stimulate IP formation at pH 6.8, however, upon exposure to more acidic pH activity can be restored. The proton concentration – response curves appears shifted to the right, i.e. sensitivity towards the ligand is reduced. In no case an increased pH-independent basal activity can be observed. A straightforward explanation for the involvement of H169 in proton sensing is difficult based on our model, given its position in extracellular loop 2, the geometry of which can not be well predicted at this stage. Mutation of H245 is not tolerated, leading to a severe, albeit not total, loss of function. According to our model this amino acid is located in helix VI facing the lipid environment, and may be required for overall structural integrity. In order to generate a receptor with a minimal number of histidines and still functional in the pH range of 7 – 7.8, we prepared and tested a H89,159,175F construct. The encoded protein indeed still functions as the wild type receptor in transiently transfected HEK cells, bottom right.

Pairs of histidines are able to co-ordinate Zn^{2+} and Cu^{2+} atoms, and this fact has been used to study GPCR structure-function (Elling et al., 1995, Nature 374, 74-77). Based on our model we expect Zn^{2+} and Cu^{2+} to inhibit proton-dependent receptor activation, by stabilising the unprotonated state of the H20-H269 pair and possibly the H17-H84 pair. Indeed, micromolar concentrations of both ions strongly inhibit OGR1-dependent IP formation stimulated at pH 6.9. In control CCL39 cells, Cu^{2+} remains without effect on thrombin-stimulated IP formation, Zn^{2+} ions led to a partial inhibition of this response.

<u>Example 3: RT-PCR expression profiling of OGR1</u>

RT-PCR expression profiling: Total RNA is prepared from cell cultures using the acid phenol method. RNA is DNAse-treated and reverse-transcribed using Superscript II (Life

technologies). Parallel reactions for OGR1 and glyceraldehydes-3-phosphate dehydrogenase (GDPH) are set up with Expand High Fidelity Taq (Roche, Switzerland) using the following temperature cycling protocol: 30 sec denaturation at 94°C, 45 sec annealing at 65°C (OGR1) or 55°C (GPDH), 50 sec extension at 72°C; 36 cycles for OGR1, 30 cycles for GPDH. GPDH is measured as internal standard for mRNA quantity. For OGR1, PCR reaction products are cloned and verified by sequencing. The following primers were used: OGR1 forward: 5'- CTGAGCCCATGAGGAGTGTG -3', reverse: 5'-GGTAGGACGCCAGCAGCAGG -3' ; GPDH forward: 5'-TTAGCACCCCTGGCCAAGG-3', reverse: 5'-CTTACTCCTTGGAGGCCATG-3'

Expression profiling by RT-PCR reveals the presence of mRNA for OGR1 in MG63 human osteosarcoma cells, and indeed we find that these cells respond strongly to neutral or acidic pH with IP formation. The signal is comparable in amplitude to that elicited by Bradykinin, which activates endogenous receptors in MG63 cells (Brechter et al., 2002, Regul. Pept. 103, 39-51). Halfmaximal activation is observed at pH 7.46 +/- 0.01 (mean +/- sem; N=5). IP formation is insensitive to PTX pretreatment and is inhibited by micromolar concentrations of copper ions, which recapitulates the results described above for ectopic expression of OGR1 in fibroblasts. Similar results are obtained for primary human osteoblast precursors isolated from trabecular bone. Half-maximal activation in primary cells occurs at pH 7.41 +/- 0.02 (mean +/- sem; N=3).

Example 4: Immunolocalisation studies.

Recombinant receptors are detected using a FITC-labelled anti-myc antibody (Zymed, No. 132511). To detect the plasma membrane marker annexin V, an alexa-labelled antibody (Molecular Probes, No. A13202) is used. Nuclei are stained with the dye H33258 (Sigma). Experiments on bone tissue are performed on 4µm sections of paraffin-embedded organs collected from 6 month old female Wistar rats. Sections are deparaffinized in xylol and antigens unmasked using pepsin digestion (10 min, Sigma). Endogenous peroxidase is blocked by a 5 minute incubation with 3% hydrogen peroxide followed by 10% goat serum for 1 hour. Immunohistochemical detection is performed using a rabbit polyclonal antibody (1:100, 3h incubation) developed by Lifespan Biosciences Inc. (Seattle) directed against the peptide epitope CFVSETTHRDLARLRG (SEQ ID NO: 2), which is identical on human and rat OGR1. Staining is revealed using the ABC peroxidase staining Kit (Santa Cruz Biotechnology).

Immunohistochemistry on rat bone sections (as described in the above paragraph) located OGR1 in osteoblasts and osteocytes.

Example 5: OGR-1 knock-out mice

Generation of OGR-1 knock-out mice:

Generation of a targeting vector for homologous recombination: The mouse OGR-1 transcript mCT51440 was identified in the mouse genome Celera database to correspond to a locus on mouse chromosome 12 designated as mCG51257. Primers are designed according to the Celéra sequence information to amplify genomic DNA used for the generation of a targeting vector for homologous recombination and knock-out of the OGR-1 gene. Sequences of primers and conditions for the amplification of two flanking genomic regions are:

For region 1: forward primer: TS145: CTATCTGCATGTGGAGCCCC and reverse primer: TS140: CTGGCAGGATAGGTCACCAT. PCR is performed using the KOD Hot Start DNA polymerase (Novagen, Germany) in a T3 PCR Biometra thermocycler. In short, 200 ng 129Sv genomic DNA are prepared in a total volume of 50 µl together with 200 µM dNTP mix, 600 nM forward primer, 600 nM reverse primer, 5 µl 10X PCR buffer (1mM $MgSO_4$) and 1 µl KOD Hot Start DNA polymerase. Settings for the amplification of genomic DNA were 94°C for 3 min., followed by 35 cycles of 94°C for 30 sec., 58°C for 30 sec., 68°C for 5 min, followed by a final extension at 68°C for 5 min. Finally, the reaction is cooled down to 4°C. 4 ul of the PCR product are subcloned using the Zero blunt Topo PCR cloning kit (Invitrogen Life Technologies, USA) according to the manufacturers instructions resulting in pTOPO-region1 which is confirmed by sequencing.

For region 2: forward primer: TS143: GCTTGCATGGTGGCTGTCTC and reverse primer: TS142: TACAACACCACCTGCACAGA. PCR is performed in a PE9600 PCR thermocycler (Perkin Elmer, USA) with Pfu DNA polymerase (Promega, USA). Briefly, 200ng of 129Sv genomic DNA are prepared in 50ul together with 200uM dNTP, 1uM forward primer and 1uM reverse primer, 1X Pfu DNA polymerase buffer (containing 2mM $MgSO_4$), 5% Dimethylsulfoxide (DMSO) and 1.25 u Pfu DNA polymerase. Settings for the touch down amplification of the genomic DNA are 94°C for 3 min., 10 cycles of 94°C for 30 sec., 68°C (-1°C/cycle) for 30 sec., 68°C for 5 min, followed by 25 cycles of 94°C for 30 sec., 55°C for 30 sec., 68°C for 5 min and a final step at 68°C for 10 min. Finally, the reaction is cooled down to 4°C. 1ul of this PCR reaction is amplified by nested PCR using forward primer TS167: CCATCGATGCTTGCCTCTAAACTAGTCT and reverse primer TS168:

ATAGTTTAGCGGCCGCCTCTACTGTCCTTGTGGCTT with Pfu polymerase under the same conditions as above. 4 ul of the PCR product are subcloned using the Zero blunt Topo PCR cloning kit (Invitrogen) according to the manufacturers constructions and confirmed by sequencing. For the generation of the targeting vector for homologous recombination a genomic fragment 1 is amplified using region 1 as template and primers forward: TS170: CCCAAGCTTAGAGCAGGTGACTGTGCATA and reverse: TS171: CCGCTCGAGCTTTGGGCCAGAAGGAGCCT. 10 ng of pTopo-region 1 are used as template in the PCR mix which is as described for the amplification of region 1 using KOD Hot Start polymerase. PCR is performed in a T3 PCR Biometra thermocycler and settings are : 94°C for 2 min., followed by 31 cycles of 94°C for 15 sec., 58°C for 30 sec., 74°C for 1 min 30 sec, followed by a final extension at 74°C for 1 min. The amplified PCR fragment is purified using the PCR purification kit (Qiagen, Germany) according to the manufacturers instructions, digested with the restriction enzymes HindIII and XhoI, ligated into the HindIII /XhoI digested vector pRAY-2 (Accession number U63120), and confirmed by sequencing. A second genomic fragment is amplified using 1 µl pTopo-region 2 as template and forward primer TS167 as well as reverse primer TS168. PCR mix is as described above for the amplification of region 1 and settings are : 94°C for 2 min., followed by 35 cycles of 94°C for 15 sec., 55°C for 30 sec., 68°C for 1 min 30 sec, followed by a final extension step 68°C for 5 min. The amplified PCR fragment is subcloned using the Zero blunt Topo PCR cloning kit and confirmed by sequencing. After digestion using the restriction enzymes ClaI and NotI, the PCR fragment is ligated into the ClaI /NotI digested vector pRAY-2 containing fragment 1. The resulting OGR-1 targeting vector is confirmed by sequencing.

ES cell culture and transfection: The final OGR-1 targeting vector is linearized using the restriction enzyme ScaI and 17 µg are electroporated into 1.5 x 10e7 129S3 mouse embryonic stem cells (ES cells) at 250 V and 500 µF. The cells are cultured in 6 cm dishes containing primary inactivated embryonic fibroblast cells. Selection medium containing G418 (200 µg/ml, Gibco, Germany) is added 24 h after electroporation. Resistant ES cell colonies are isolated 10 days after electroporation and analyzed by nested PCR to identify homologous recombination events of the targeting construct into the OGR-1 locus (genotyping by PCR).

Genotyping by PCR: ES cells are extracted in 50 µl lysis buffer (0.05% SDS, 50 µg/ml proteinase K, 10 mM Tris/HCL, pH7.4) and diagnostic PCR is performed using 1 µl crude ES cell extract in a total volume of 25 µl together with 200 µM dNTP mix, 600 nM forward primer, 600 nM reverse primer, 1x Taq PCR master mix (Qiagen), in a Tgradient PCR Biometra thermocycler. Primers used for PCR are forward: TS207: TGATATTGCTGAAGAGCTTGGCGGC and reverse: TS203: CCAGGGTAGCTTTGCAACATGC for the first amplification as well as forward: TS208: AGCGCATCGCCTTCTATCGCC and reverse: TS204: ATGGGCTTTGCCATGAGGCAG for the nested reaction. PCR conditions are 95°C for 3 min; 35 cycles of 95°C for 30 sec; 62°C for 45 sec; 72°C for 2 min. For the nested reaction 1 µl of the first reaction are amplified at 95°C for 3min; 25 cycles of 95°C for 30 sec; 62°C for 45 sec; 72°C for 2 min. Finally, 10 µl of the nested PCR reaction are analysed on a 1% agarose gel.

Southern genotyping of ES cells: In order to generate a probe suitable for Southern hybridization, a OGR-1 genomic region is amplified by PCR using 100 ng pTopo-region 1 as template. Primers used for amplification are forward: TS146: CAAGGGCAGGGGAGTCAAGG and reverse: TS159: TAATTATTCTACTTTATTAC. The PCR mix was as described above using Pfu DNA polymerase. Settings for PCR were 94°C for 2 min., 10 cycles of 94°C for 15 sec., 55 (-1°C/cycle) for 15 sec., 72°C for 30 sec, followed by 25 cycles of 94°C for 15 sec., 45°C for 15 sec., 72°C for 30 sec. Finally, the reaction was cooled down to 4°C. The amplified PCR fragment was purified as described above using the PCR purification kit.
Genomic DNA from 129S3 ES cells is digested over night using 12 µg genomic DNA and the restriction enzymes SspI/RsrII or SspI/XhoI. The digested DNA is separated on a 0.9% agarose gel and blotted to a Hybond N+ membrane (Amersham). Random prime labelling of the DNA probe with ^{32}P-dCTP is performed using the Amersham rediprime II kit (Amersham, UK) as described by the manufacturer. Membranes are hybridized over night at 65°C in PerfectHyb Plus hybridization buffer (Sigma-Aldrich, Germany), washed in 0.5 x SSC; 0.1% SDS and imaged by exposure to a Kodak X-O-Mat film.

A neo probe corresponding to the NheI/BamHI fragment of pRAY2 vector is used under the same conditions to confirm the unique integration of the targeting construct.

Karyotype analysis: ES cells are splitted the day before chromosome spreads are performed. For chromosome spreading, ES cells are treated with 10 µg/ml Colchicin (KaryoMax, Gibco) for 4.5 h at 37°C and 10% CO_2 followed by an incubation for 10 min at room temperature in prewarmed (37°C) 0.56% KCl. Fixation is performed by using ice cold methanol/acetic acid (3:1). Spreading of the chromosomes is analyzed on a microscope.

Blastocyste injection and breeding: Targeted ES cells are injected into C57Bl/6 host blastocysts and transferred into pseudopregnant C57Bl/6 x Balb/c N1 foster mothers. Chimeric offspring are identified by coat pigmentation. *Male chimeras are mated with 129S3 wildtype females. The offspring are tested for germline transmission by genotyping PCR.*

<u>Example 5: pH dependent stimulation of intracellular calcium transients (ligand assay):</u>
Method: Cytoplasmic calcium transients are recorded using the calcium indicator Fluo-3 [28] and a fluorescence-imaging plate reader (Molecular Devices). Cells are loaded with the acetoxymethylester of the dye (2 µg/ml) for 1 h at 37°C in full medium containing 5 mM probenicide to inhibit the multidrug resistance transporter. Cells are then washed and maintained in PSS buffered with 5 mM HEPES at pH 7.6 for 45 min at room temperature, and maintained at room temperature for the rest of the experiment. After transfer to the reader a baseline is recorded (Fb), and cells are then stimulated by addition of an appropriate amount of stronger buffer. The fluorescence readout F is normalised calculating F' = (F - Fb) / Fb.

Result: Shift of extracellular pH from pH 7.6 to more acidic values results in rapid and transient elevations of intracellular calcium concentration, indicating release of the cation from intracellular stores. The amplitude of the response is pH – dependent, increasing with acidity. Half-maximal activation occurs at pH 7.20 +/- 0.04 (mean +/- sem; N=3),

SEQUENCE LISTING

<110> Novartis AG

<120> Novel G-Protein Coupled Receptors and DNA Sequences Thereof

<130> 4-33201P1

<160> 2

<170> PatentIn version 3.1

<210> 1

<211> 365

<212> PRT

<213> Homo sapiens

<220>

<221> PEPTIDE

<222> (1)..(365)

<223>

<400> 1

Met Gly Asn Ile Thr Ala Asp Asn Ser Ser Met Ser Cys Thr Ile Asp
1 5 10 15

His Thr Ile His Gln Thr Leu Ala Pro Val Val Tyr Val Thr Val Leu
20 25 30

Val Val Gly Phe Pro Ala Asn Cys Leu Ser Leu Tyr Phe Gly Tyr Leu
35 40 45

Gln Ile Lys Ala Arg Asn Glu Leu Gly Val Tyr Leu Cys Asn Leu Thr
50 55 60

Val Ala Asp Leu Phe Tyr Ile Cys Ser Leu Pro Phe Trp Leu Gln Tyr
65 70 75 80

Val Leu Gln His Asp Asn Trp Ser His Gly Asp Leu Ser Cys Gln Val
85 90 95

Cys Gly Ile Leu Leu Tyr Glu Asn Ile Tyr Ile Ser Val Gly Phe Leu
100 105 110

Cys Cys Ile Ser Val Asp Arg Tyr Leu Ala Val Ala His Pro Phe Arg
115 120 125

Phe His Gln Phe Arg Thr Leu Lys Ala Ala Val Gly Val Ser Val Val
130 135 140

Ile Trp Ala Lys Glu Leu Leu Thr Ser Ile Tyr Phe Leu Met His Glu
145 150 155 160

Glu Val Ile Glu Asp Glu Asn Gln His Arg Val Cys Phe Glu His Tyr
165 170 175

Pro Ile Gln Ala Trp Gln Arg Ala Ile Asn Tyr Tyr Arg Phe Leu Val
180 185 190

Gly Phe Leu Phe Pro Ile Cys Leu Leu Leu Ala Ser Tyr Gln Gly Ile
195 200 205

Leu Arg Ala Val Arg Arg Ser His Gly Thr Gln Lys Ser Arg Lys Asp
210 215 220

Gln Ile Gln Arg Leu Val Leu Ser Thr Val Val Ile Phe Leu Ala Cys
225 230 235 240

Phe Leu Pro Tyr His Val Leu Leu Leu Val Arg Ser Val Trp Glu Ala
245 250 255

Ser Cys Asp Phe Ala Lys Gly Val Phe Asn Ala Tyr His Phe Ser Leu
260 265 270

Leu Leu Thr Ser Phe Asn Cys Val Ala Asp Pro Val Leu Tyr Cys Phe
275 280 285

Val Ser Glu Thr Thr His Arg Asp Leu Ala Arg Leu Arg Gly Ala Cys
290 295 300

Leu Ala Phe Leu Thr Cys Ser Arg Thr Gly Arg Ala Arg Glu Ala Tyr
305 310 315 320

Pro Leu Gly Ala Pro Glu Ala Ser Gly Lys Ser Gly Ala Gln Gly Glu
325 330 335

Glu Pro Glu Leu Leu Thr Lys Leu His Pro Ala Phe Gln Thr Pro Asn
340 345 350

Ser Pro Gly Ser Gly Gly Phe Pro Thr Gly Arg Leu Ala
355 360 365

<210> 2

<211> 16

<212> PRT

<213> Artificial

<220>

<221> PEPTIDE

<222> (1)..(16)

<223>

<400> 2

```
Cys Phe Val Ser Glu Thr Thr His Arg Asp Leu Ala Arg Leu Arg Gly
1               5                   10                  15
```

Claims

1. The use of an isolated polypeptide in the manufacture of a medicament for disease and medical conditions in which proton homeostasis is altered; said polypeptide is selected from one of the groups consisting of:
(a) an isolated polypeptide encoded by a polynucleotide comprising the sequence of SEQ ID NO: 1;
(b) an isolated polypeptide comprising a polypeptide sequence having at least 80% identity to the polypeptide sequence of SEQ ID NO: 1;
c) an isolated polypeptide having at least 80% identity to the polypeptide sequence of SEQ ID NO: 1; and
d) the polypeptide sequence of SEQ ID NO: 1;
(e)fragments and variants of such polypeptides in (a) to (d) which show an inhibitory effect of the pH dependent Inositol phosphate formation in CCL39 hamster fibroblast cells; and
(f) polypeptides in (a) to (c) which show a pH dependent Inositol phosphate formation in CCL39 hamster fibroblast cells.

2. The use of the isolated polypeptide as claimed in claim 1, said polypeptide comprising the polypeptide sequence of SEQ ID NO: 1.

3. The use of the isolated polypeptide as claimed in claim 1, said polypeptide consisting of the polypeptide sequence of SEQ ID NO: 1.

4. The use of an isolated polynucleotide for the manufacture of a medicament for disease and medical conditions in which proton homeostasis is altered; said polynucleotide is selected from one of the groups consisting of:
(a) an isolated polynucleotide comprising a polynucleotide sequence having at least 80% identity to the polynucleotide sequence with accession number NM_003485;
(b) an isolated polynucleotide having at least 80% identity to the polynucleotide with accession number NM_003485;
(c) an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide sequence having at least 80% identity to the polypeptide sequence with accession number NM_003485;

(d) an isolated polynucleotide having a polynucleotide sequence encoding a polypeptide sequence having at least 80% identity to the polypeptide sequence with accession number NM_003485;
(e) an isolated polynucleotide with a nucleotide sequence of at least 100 nucleotides obtained by screening a library under stringent hybridization conditions with a labeled probe having the sequence with accession number NM_003485 or a fragment thereof having at least 15 nucleotides;
(f) a polynucleotide which is the RNA equivalent of a polynucleotide of (a) to (e); or a polynucleotide sequence complementary to said isolated polynucleotide and polynucleotides that are variants and fragments of the above mentioned polynucleotides or that are complementary to above mentioned polynucleotides, over the entire length thereof; and
(g) polynucleotides of (a) to (e) comprising a polynucleotide sequence encoding a polypeptide sequence which shows a pH dependent Inositol phosphate formation in CCL39 hamster fibroblast cells.

5. An expression system comprising a polynucleotide capable of producing a polypeptide of claims 1 to 3 when said expression vector is present in a compatible host cell.

6. A recombinant host cell comprising the expression vector of claim 5 or a membrane thereof expressing the polypeptide of claims 1 to 3.

7. A process for producing a polypeptide of claims 1 to 3 comprising the step of culturing a host cell as defined in claim 6 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

8. A fusion protein consisting of the Immunoglobulin Fc-region and any one polypeptide of claims 1 to 3.

9. An antibody immunospecific for the polypeptide of any one of claims 1 to 3.

10. An antibody or a fragment thereof which specifically binds to a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2 or a fragment of a polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:2.

11. A method for screening to identify compounds that stimulate or inhibit the function or expression level of the polypeptides of claims 1 to 3 comprising a method selected from the group consisting of:

(a) measuring or, detecting, quantitatively or qualitatively, the binding of a candidate compound to the polypeptide (or to the cells or membranes expressing the polypeptide) or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound;

(b) measuring the competition of binding of a candidate compound to the polypeptide (or to the cells or membranes expressing the polypeptide) or a fusion protein thereof in the presence of a labelled competitor;

(c) testing whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide, using detection systems appropriate to the cells or cell membranes expressing the polypeptide;

(d) mixing a candidate compound with a solution containing a polypeptide of any of the polypeptides of claims 1 to 3, to form a mixture, measuring activity of the polypeptide in the mixture, and comparing the activity of the mixture to a control mixture which contains no candidate compound; or

(e) detecting the effect of a candidate compound on the production of mRNA encoding said polypeptide or said polypeptide in cells, using for instance, an ELISA assay, and

(f) producing said compound according to biotechnological or chemical standard techniques.

Abstract:
This invention relates to the newly identified use of certain G protein-coupled receptors (GPCRs), in particular to the Ovarian cancer G protein-coupled receptor 1 (OGR1) polypeptides, and polynucleotides encoding such GPCR polypeptides, to their use in diagnosis and in identifying compounds that are agonists, antagonists to said GPCRs, and to the production of such polypeptides and polynucleotides.